Next-Generation Sequencing to Guide Clinical Trials

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Abstract

Rapidly accruing knowledge of the mutational landscape of malignant neoplasms, the increasing facility of massively parallel genomic sequencing, and the availability of drugs targeting many "driver" molecular abnormalities have spurred the oncologic community to consider how to use these new tools to improve cancer treatment. In order to assure that assignment of patients to a particular targeted treatment is likely to be beneficial to the patient, it will be necessary to conduct appropriate clinical research. It is clear that clinical (histology and stage) eligibility criteria are not sufficient for most clinical trials using agents that target mutations that are present in only a minority of patients. Recently, several clinical trial designs have been suggested to test the benefit of targeted treatment in molecular and/or clinical subgroups of patients. However, challenges remain in the implementation of such trials, including choice of assay, levels of evidence regarding gene variants, tumor heterogeneity, identifying resistance mechanisms, the necessity of screening large numbers of patients, infrastructure needs, and collaboration of investigators and industry. This article reviews current trial designs and discusses some of the considerations, advantages, and drawbacks of designing clinical trials that depend on particular molecular variants as eligibility criteria. *Clin Cancer Res; 21(20); 4536–44. ©2015 AACR.*

See all articles in this *CCR Focus* section, "Innovations to Speed Drug Development."

Introduction

Recent advances in the systematic massive parallel (next-generation) sequencing of cancer genomes have revealed that individual tumors frequently harbor driver somatic mutations and other aberrations that confer growth advantage and positive selection (1). The conventional diagnostic and therapeutic paradigms in oncology are primarily based on histopathologic information, supplemented by a limited panel of molecular profiling tests in some tumor types that may be used to guide cancer treatment decisions, such as *HER2* testing in breast cancer and *KRAS* genotyping in colorectal cancer. The increasing accessibility of high-throughput genotyping and genomic sequencing at point-of-care, coupled with the era of molecularly targeted therapeutics, have led to growing efforts to match patients with druggable genetic aberrations to specific targeted agents with the hope of achieving greater efficacy than treatment selection based on empiricism. Retrospective series evaluating the success of such matching strategies have reported mixed outcomes (2, 3). However, there are limitations to these retrospective analyses, including the potential subjectivity of how "matching" is defined and the imbalance of confounding factors that may affect outcome.

Prospective large-scale molecular profiling programs have been established in cancer centers throughout many parts of the world (4, 5), with some of these operating as part of extensive national efforts (6, 7). In addition, there is a rapid development of commercial vendors that perform genomic characterization of patients' tumor samples on a fee-for-service basis (8, 9), although to date no next-generation sequencing (NGS) test has been cleared or approved by the FDA for this purpose. However, much information about the clinical validity of molecular abnormalities, as well as the clinical utility of these tests remains unknown (see Text Box 1). With an increasing number of patients whose tumors have undergone molecular evaluation, the need to translate these findings into clinically relevant outcomes becomes pressing. The potential sources of drug access to enable genotype-target matching in the oncology setting include the on-label or off-label prescription of marketed agents, as well as through the design of clinical trials that provide a channel to approved or investigational compounds. However, off-label use in clinical practice does not add to the body of knowledge regarding whether or not a given variant in a given histologic type of tumor will be predictive of clinical benefit from a targeted agent. Additionally, as the majority of individual platforms have not undergone extensive validation with review from the regulatory agencies, and the extent of profiling needed for treatment decision making in many scenarios is still under debate, it is difficult to identify appropriate treatments based on a given molecular profile for which there are limited data. When multiple molecular aberrations exist within the context of a "read-out" or report, it is often difficult to define the most relevant target mutation or pathway. There are limited data suggesting that, in select scenarios of single-drug administration, targeting only one molecular aberration could have a negative impact on patient outcome due to potential upregulation of alternative pathways (10, 11). Even when there are multiple aberrations that might be potentially actionable, the ability to use drug combinations is currently limited due to multiple factors, including overlap in toxicity seen with certain drug combinations or the potential need to study more than two molecularly targeted agents in combination. Treating patients anecdotally based on an available molecular profile may not be

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Text Box 1. Glossary for Commonly Used Terms in the Cancer Genomics Era

Analytical validation: assures the assay can detect the desired molecular change accurately.

Clinical validation: assures there is a relationship between the result of the assay and the clinical outcome of interest (e.g., type of tumor, response, and survival). The calculations for positive predictive value (i.e., number of true positives divided by total number of positive results) and negative predictive value (i.e., number of true negatives divided by total of negative results) are typically included in clinical validation.

Clinical utility: use of the assay results in better outcomes for the patient than not using the assay.

Integral marker: biomarker that must be measured in every patient on a clinical trial in order for the trial to proceed; e.g., biomarker is used for eligibility or stratification.

Integrated marker: biomarker is measured in all or most of the patients on the trial, but will not be used to determine treatment on the trial. May be used for clinical validation or assessment of clinical utility of the marker for the benefit of future patients.

Depth of sequencing coverage: how many times a particular DNA fragment is sequenced.

Ploidy: the number of single sets of chromosomes in a cell.

Prognostic biomarker: predicts clinical course regardless of therapy.

Predictive biomarker: predicts outcome for a specific agent (e.g., $BRAF^{V600E}$ and vemurafenib or dabrafenib).

Purity: an estimation of how much of the tumor's genome sequence is different from that of the normal matched tissue as a result of the presence of mutation(s).

in the patients' best interest and could also limit their subsequent eligibility for clinical trials. A new generation of genomic-based clinical trials has emerged to maximize the opportunity to allocate treatment based on patients' tumor molecular profiles and to provide information on the clinical validity and utility of assigning treatment based at least in part on molecular profiling.

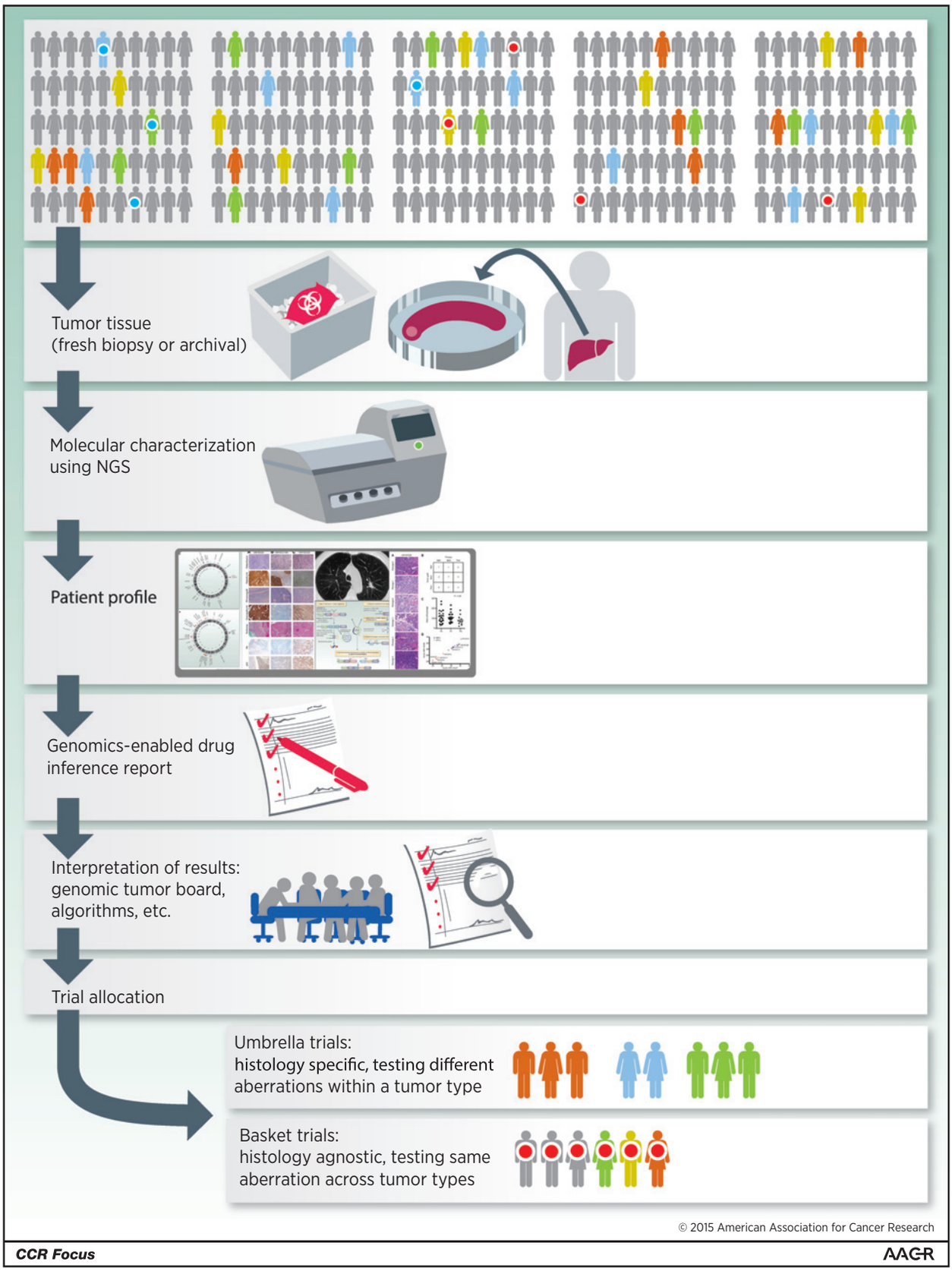
Using NGS to Identify Patients for Clinical Trials

Although the application of NGS technologies as a screening strategy to identify patients for genomic-based clinical trials has gained acceptance in the oncology community (Fig. 1), this approach has many complexities that must be considered. There is no consensus on whether archived tumor or fresh biopsy should be selected for molecular profiling. A freshly procured tumor sample is theoretically preferred over archived, formalin-fixed, paraffin-embedded diagnostic tissue, despite easier access of the latter, as archived tissue may not have been preserved using optimal processes and may not reflect the current molecular landscape due to clonal evolution (12, 13). However, core needle biopsies, especially of deep visceral organs, can present risk to the patient, incur additional costs, and require operational infrastructure to organize if these procedures are being done in a large number of patients with minimal delay. For both archived tissue and fresh biopsy, there is uncertainty as to whether a single sample reflects the entire tumor genomic heterogeneity. Although liquid biopsies (blood and plasma) hold promise as a means to perform genotyping of circulating tumor DNA (ctDNA) and potentially capture most heterogeneity in tumors (14), there is no standardized routine assay in most Clinical Laboratory Improvement Amendments–certified molecular diagnostic laboratories, and most ctDNA assays currently under development are confined to one or a small number of mutations. Additionally, although there are advantages to serial sampling with ctDNA, it is felt that, to maximize data obtained from liquid biopsies, matching them initially against a fresh core biopsy for profiling assessment is important for accurate interpretation.

After the appropriate tumor specimen has been selected, the frequency with which a variant is detected depends on the depth of sequencing coverage, tumor purity, and ploidy (see Text Box 1). As tumor purity increases, so should the frequency of a somatic variant. Ploidy and focal copy number alterations will also affect the frequency with which a variant is called in the tumor population. In addition to read depth, computational predictions of tumor purity, ploidy, and subclonal structure provide important information to guide the management of each case. Variant annotation based on the curation of published literature and *in silico* functional algorithms is critical to ensure correct mutation calling. Peripheral blood germline DNA sequencing, in addition to public databases such as dbSNP and 1000 Genomes, is used by many molecular profiling programs to exclude known SNPs and to refine the variant annotation process (15, 16).

In the design of genomic-based clinical trials, there must be clear guidance on molecular selection criteria to avoid any ambiguities. Ideally, eligibility instructions should provide guidance at a variant level with details related to the inclusion or exclusion of variants of unknown or uncertain significance. Furthermore, in cases with multiple somatic variants identified in a tumor, a hierarchical prioritization scheme should be described based on prespecified rules such as known functionality or allele frequency. Variant- and gene-level data need to be tracked during the conduct of genomic-based clinical trials and reported in related publications (e.g., as supplemental materials) to optimize the interpretation of such results across studies.

Genomic aberrations that are used as integral biomarkers (see Text Box 1) for clinical trial eligibility or stratification should be ascertained by assays that have undergone rigorous standardization and quality assurance evaluation performed in certified diagnostic laboratories. The majority of genomic-based clinical trials that are ongoing or currently planned have established centralized laboratories or molecular characterization hubs to perform trial-specific biomarker assays. Some of these trials have built in directly linked molecular prescreening strategies to seek out specific genotypes for study enrollment. Others have advocated the separation of molecular prescreening efforts from single therapeutic trials such that profiled patients may have options for enrollment into one of multiple trials that select for the aberrations of interest, as opposed to fixed allocation to only one individual trial (17). As these next-generation genomic-based clinical trials are launched and accumulate experience, best practices can be gleaned from exemplars to help set references and benchmarks.



Genomic-Based Clinical Trials

In the new genomics era, it is hoped that the traditional approach to early clinical cancer drug development can be made more efficient and effective, and lead to larger improvements in efficacy. The current therapeutic armamentarium, although beneficial to some patients, is still insufficient for many. The myriad number of possible mutations in critical molecular pathways necessitates a broad range of agents to target them. As the number of agents increases, the current approach of solely using histologic interpretation for defining treatment options is inadequate and must take into consideration underlying tumor genomics and biology. It has become increasingly obvious that more and better therapies, as well as efficient means of identifying which therapies will match best with specific patients as early as possible in drug development are needed to optimally identify major impacts in the treatment of cancer. Other articles in this *CCR Focus* also address key limitations in the current drug development process and provide suggestions to expedite the evaluation and delivery of the best treatments to patients with cancer in a safe, efficient, and value-based manner (18–21).

Novel clinical trial designs employing the latest techniques in molecular profiling are now being used early in drug development, sometimes in the dose finding stage, to inform therapeutic choices for patients with cancer. Classical nonselective recruitment to early phase trials may be questioned as to whether such recruitment is the best use of limited financial and patient resources. Additionally, for low prevalence mutations, to obtain a therapeutic signal with a targeted agent in such a trial would require screening large numbers of patients for the mutation, reducing the effectiveness of such protocol recruitment strategies. If sufficiently strong scientific background and preclinical data are available to support an enrichment strategy, patient selection based on tumor genomic profiles may be applied in the dose escalation and/or cohort expansion stages of phase I trials. Innovative ways to determine optimal dosing for patients enrolled, based on their genotype, are actively being sought, as treatment at the maximal tolerated dose may not be needed in the presence of oncogenic addiction or dependence. Further, adaptive methodologies to optimize genotype-target matching can be useful in these trials to identify early efficacy signals and inform the planning of later phase studies.

"Basket" trials (Table 1) are genotype-focused designs involving the testing of a single drug on a specific mutation or mutations in a variety of cancer types (22–24). This design tests response in the presence of a particular molecular biomarker in different tumor histologies, but sometimes also has an "any tumor" basket, accepting different tumor types (especially rare histologies) possessing the eligible molecular abnormality. Each of these cohorts is analyzed separately but in a single clinical trial. If the treatment under study demonstrates a signal of efficacy in a particular cohort, the cohort can be expanded to enroll more patients of that particular disease type. Conversely, cohorts that do not demonstrate efficacy can be closed while the study continues with other disease types. A basket trial design is especially advantageous when the mutation or cancer type is rare, as it allows flexibility for a combination of multiple, independent, small-scale phase II studies within a single trial. The intent of basket trials can be either exploratory or for registration, although currently there are few known examples of the latter. One example of a basket trial designed with registrational intent was the B2225 trial, a broad study of imatinib in malignant diseases with activation of any imatinib-targeted kinase, this strategy was used for FDA regulatory approval of imatinib for several rare conditions (25).

"Umbrella" trials (Table 2) involve the testing of different drugs targeting different mutations either in a single cancer subtype or in a variety of cancer subtypes (24). These studies typically utilize an individualized treatment plan formulated after analysis of the molecular profile of each patient's tumor. A preassembled portfolio of treatments is used with a refined molecularly guided decision tree or algorithm.

"Hybrid" trials (Table 3) represent a mix of "umbrella" and "basket" trial design frameworks such that under the auspice of one protocol, there are either multiple "umbrella" subtrials (same histology, different molecular aberrations), or multiple "basket" subtrials (same molecular aberrations, different histologies) recruiting patients.

The current clinical drug development landscape is characterized by the emergence of many basket and umbrella trials (Tables 1 and 2), and some hybrid trials (Table 3). However, the majority of these trials are limited in that they only have monotherapy arms. To maximize the potential benefits of genomic-based trials, additional advances in experimental therapeutic strategies, particularly of drug combinations, are needed. Approved targeted drug combinations are currently uncommon, yet many tumors have multiple genomic aberrations potentially requiring "cocktails" of several targeted agents, in combination, for potential maximum efficacy. Tumors of the same histologic type may also have different comutations, requiring individualization of combinations for patients with the same histologic tumor type. There are additional challenges to targeted drug combinations including, at minimum, overlapping drug toxicities, and the requirement for multiple sponsors working together. Finally, drug ratios for each agent in combination, as well as appropriate dosing and scheduling needed for each individual's tumor may be different based on the pharmacokinetic and pharmacodynamic requirements for tumor target inhibition. Comprehensive solutions to these challenges are currently unknown.

Statistical Considerations for Genomic-Based Clinical Trials

When designing any of these types of trials, consideration will need to be given to the choice of primary outcome measure (e.g., progression-free survival and overall response rate), whether the trial will use a comparator arm (e.g., standard treatment, other) and whether patients whose molecular profiles do not "match" one of the genotype-target–based treatments should nevertheless receive a "catch-all" treatment on the trial. Such trials generally use enrichment designs (only patients with the required molecular

Figure 1.
Application of NGS to guide clinical trials in cancer patients. First, tumor tissue (either archived tumor specimen or freshly procured tumor biopsy) is subjected to molecular characterization using NGS and/or other technologies. After appropriate annotation, a report detailing the molecular profiling results is generated. Interpretation of the results may be done using predefined algorithms and/or via molecular tumor board discussions. Based on the results, patients may be recommended for specific clinical trial allocation, such as enrollment into genomic-based clinical trials (e.g., umbrella, basket, or hybrid trials).

Table 1. Examples of active umbrella trials matching patients to therapies based on molecular profiles

Program name	Lead organization	Design	Histology	Indication	# Expected to accrue	Primary outcome measure(s)	Clinicaltrials.gov identifier
ALCHEMIST	US National Cancer Institute	Enrichment, research	Stage IB–IIIA lung adenocarcinoma	Screening	8,000	Feasibility, genotyping for placement on adjuvant trials	NCT02194738
	ECOG-ACRIN	R	Stage IB–IIIA adenocarcinoma of lung, with ALK fusion	Adjuvant	378	OS	NCT02201992
	ALLIANCE	R	Stage IB–IIIA adenocarcinoma of lung, with activating EGFR mutation	Adjuvant	450	OS	NCT02193282
BATTLE-2	MD Anderson	A–R	NSCLC	Metastatic	450	8-Week DCR	NCT01248247
FOCUS 4	Cancer Research UK	R	Colorectal	Metastatic	Variable (maximum 2,329)	PFS	EudraCT# 2012-005111-12 (37)
GEMM	Yale University	R	Advanced non-V600–mutated metastatic melanoma	Metastatic	96	BORR	NCT02094872
ISPY-2	Quantum Leap Healthcare Collaborative	A–R	Locally advanced breast cancer	Neo-Adjuvant	800	pCR	NCT01042379
LUNG-MAP	SWOG and NCTN	R	Squamous	Metastatic	10,000 (screening)	PFS	NCT02154490
SAFIR-02 breast	UNICANCER	R	Metastatic non-$HER2^{+}$ breast cancer	Metastatic	400 (screening) 210 (randomized)	PFS	NCT02299999
SAFIR-02 lung	UNICANCER	R	NSCLC	Metastatic	650 (screening) + 220 (treatment)	PFS	NCT02117167

Abbreviations: A-R, adaptively randomized; ALLIANCE, Alliance for Clinical Trials in Oncology; BORR, best overall response rate; DCR, disease control rate; ECOG-ACRIN, Eastern Cooperative Oncology Group and the American College of Radiology Imaging Network; NCTN, National Clinical Trials Network; NR, nonrandomized; NSCLC, non–small cell lung cancer; OS, overall survival; pCR, rate of pathologic complete response; PFS, progression-free survival; R, randomized; SWOG, Southwest Oncology Group.

abnormality are entered), and thus, the effect of the treatment on patients without the molecular abnormality will not be known (26, 27). Also, without a standard control group, the prognostic characteristics of the biomarker or mutation cannot be assessed. Outcome measures in the enriched population given the molecularly targeted treatment should not be compared with outcomes from standard treatment in non–biomarker-characterized patients (historical controls). This problem can be addressed by randomizing patients with the molecular abnormality of interest to either standard treatment or targeted treatment, but with the possible drawback that the trial will not be as attractive to clinicians or patients.

Tables 1–3 show respective examples of current active umbrella, basket, and hybrid trials matching patients to treatments based on molecular profiles. There has been criticism that the selected therapeutic interventions are insufficient in these trials as many of them examine targeted agents as monotherapy, thus potentially limiting efficacy, and also because many relevant genomic aberrations are yet to be effectively targeted. Many researchers are concerned that if no therapeutic benefit is identified from investigating novel targeted agents as monotherapy, enthusiasm for continued investigation of these agents in future clinical trials may be limited. Therapeutic benefit of such agents may require these agents to be used in combination, rather than as monotherapy. However, trials using the umbrella, basket, and hybrid designs have the potential to enhance our understanding of pathway interactions, cross-talk, and mechanisms underpinning lack of drug effect and/or drug resistance to better identify combinations that may best be explored to maximize treatment response and ultimately improve outcomes for patients. If used in well-controlled situations, such trials may unveil the reasons for lack of response based on additional pathway activation that is either enhanced or left unblocked by single-drug therapies, as well as defining characteristics for which monotherapy is sufficient.

Pros and Cons of Genomic-Based Clinical Trials

Compared with an "all comers" trial, genomic-based clinical trials offer some definite advantages. Chief among these are the opportunity to develop clinical evidence that targeting several particular molecular abnormalities in the same trial will reveal one or several potentially clinically beneficial treatments. An umbrella trial will allow evaluation of treatments for several molecular subclasses of patients within the same histologic tumor type. If such a trial is designed to proceed to a randomized phase III trial, as in LUNG-MAP (28, 29), it may significantly shorten time to regulatory approval in a particular disease, although effectively "weeding out" targeted agents without significant benefit, and allowing inclusion of additional "arms" as promising data

Table 2. Examples of active basket trials matching patients to therapies based on molecular profiles

Program name	Lead organization	Design	Histology	Indication	# Expected to accrue	Primary outcome measure(s)	Clinicaltrials.gov identifier
CREATE	EORTC	NR	ALK/MET activated advanced solid tumors	Metastatic	582	ORR	NCT01524926
IMPACT II	MD Anderson	R	Advanced solid tumors	Metastatic	1,362	PFS	NCT02152254
My Pathway	Genentech	NR	Advanced solid tumors	Metastatic	500	ORR	NCT02091141
SHIVA	Institut Curie	R	Advanced solid tumors	Metastatic	1,000	PFS	NCT01771458
SIGNATURE	Novartis	NR	PI3K-activated solid tumors and/or hematologic malignancies	Metastatic	145	CBR	NCT01833169
			$BRAF^{V600}$-mutated solid tumors and/or hematologic malignancies	Metastatic	12	CBR	NCT01981187
			PTCH1 or SMO mutated solid tumors and/or hematologic malignancies	Metastatic	10	CBR	NCT02002689
			RAS/RAF/MEK activated solid tumors and/or hematologic malignancies	Metastatic	110	CBR	NCT01885195
			CDK4/6 pathway activated solid tumors and/or hematologic malignancies	Metastatic	90	CBR	NCT02187783
			FGFR mutated solid tumors and/or hematologic malignancies	Metastatic	70	CBR	NCT02160041
			ALK or ROS1 mutated solid tumors and/or hematologic malignancies	Metastatic	70	CBR	NCT02186821
			Solid tumors and/or hematologic malignancies with aberrations in FGFR, PDGFR, VEGF, cKIT, FLT3, CSFR1, Trk, or RET	Metastatic	80	CBR	NCT01831726
SPECTA	EORTC	NR	Advanced colorectal cancer	Metastatic	2,600	TMA (screening)	NCT01723969
			Thoracic tumors	Any stage	3,500	TMA (screening)	NCT02214134
			Brain neoplasms	Any stage	300	TMA (screening)	NCT02307604
VE-BASKET	Hoffmann-La Roche	NR	$BRAF^{V600E}$-mutated advanced solid tumors	Metastatic	160	ORR	NCT01524978
WINTHER	WIN Consortium	NR	Advanced solid tumors	Metastatic	200	PFS	NCT01856296
Dabrafenib and trametinib in $BRAF^{V600E}$-mutated rare cancers	GlaxoSmithKline	NR	$BRAF^{V600E}$ mutation–positive tumor: including anaplastic thyroid cancer, biliary tract cancer, gastrointestinal stromal tumor, nonseminomatous germ cell tumor/nonseminomatous germ cell tumor, hairy cell leukemia, WHO grade 1 or 2 glioma, WHO grade 3 or 4 (high-grade) glioma, multiple myeloma, and adenocarcinoma of the small intestine	Advanced disease without standard treatment options	135	ORR	NCT02034110

Abbreviations: CBR, rate of clinical benefit; ECOG-ACRIN, Eastern Cooperative Oncology Group and the American College of Radiology Imaging Network; EORTC, European Organization for Research and Treatment of Cancer; NCTN, National Clinical Trials Network; NR, nonrandomized; NSCLC, non–small cell lung cancer; ORR, overall response rate; PFS, progression-free survival; R, randomized; TMA, tumor marker assessment; WIN, worldwide innovative networking.

Table 3. Examples of active "hybrid" trials matching patients to therapies based on molecular profiles

Program name	Lead organization	Design	Histology	Indication	# Expected to accrue	Primary outcome measure(s)	Clinicaltrials.gov identifier
NCI-MATCH	NCI ECOG-ACRIN and NCTN	NR	Advanced solid tumors and lymphomas	Metastatic	3,000	ORR	NCT02465060
NCI-MPACT	NCI	R	Advanced solid tumors	Metastatic	700	ORR or PFS	NCT01827384

Abbreviations: NR, nonrandomized; ORR, overall response rate; PFS, progression-free survival; R, randomized.

emerge. In LUNG-MAP, for example, the phase II arm sets an HR of 0.4 to 0.5 for progression-free survival. If this endpoint is met, the phase II trial will proceed to phase III within LUNG-MAP, with the endpoint of overall survival. In addition, presuming all molecularly characterized patients with the same histology will not respond uniformly, such trials will allow research about other genomic or clinical features that may modify or prevent response, leading to new insights about combinations with drugs that address resistance pathways. Similar advantages apply to a basket trial, in which a broad screening effort can identify patients with various tumors with the same molecular abnormality. Those cancer histologies that have relatively high prevalence of the molecular target create subgroups that may develop solid signals of efficacy within a particular histologic tumor type. Here, in addition to being able to discern clinical and molecular features that modify response, one can also look at such features across histologic tumor types. Such a trial could speed registration for a given treatment to many and not just a single tumor histologic type.

However, there are also certain challenges to genomic-based clinical trials. International collaboration may be required for all but the most common histologic tumor types and genomic mutations. Time and effort are required to set up proper infrastructure and account for different regulations across countries as well as to negotiate agreements with several pharmaceutical companies. For trials to be most efficient, high levels of evidence should exist that the chosen drug will result in benefit for tumors with a particular molecular characteristic. It is not often possible to discern "best in class" drugs, or promising drugs may still be too early in development to be included. Many molecular abnormalities have low prevalence in any given tumor type (<10%), requiring a broad screening effort, and molecular abnormalities with high prevalence (e.g., *TP53* or *RAS* mutation) may not have effective targeted regimens. Time delays may increase the risk that standard of care treatment may change during the time required to activate and/or complete the trial, or new drugs may be approved that affect the design of, or accrual to, the trial. For basket trials, the regulatory pathway may not be clear, unless the signal of activity is exceptionally strong. In rare tumors, without existing standard treatment, the regulatory path may be easier than in tumors with standard treatment. In tumors where standard treatment is of high efficacy, there is significant challenge to show improvement by a novel treatment. Finally, there is also the possibility that NGS may identify previously undiagnosed germline mutations (which may be present in tumor tissue as well), even in patients without a family history (30, 31). Best practices to address these issues have yet to be worked out.

Future Directions and Conclusions

Inspired by the recent approval and high activity of targeted agents against driver mutations in cancers formerly considered "untreatable," the desire to use genomic sequencing to improve treatment for all cancer patients is strong. Currently, patient tumors can be sent for sequencing with the hope that some treatment of currently unknown efficacy in that tumor type may be beneficial. The challenge for the clinical research community is to acquire the data showing this practice can be clinically beneficial (clinical utility). Use of NGS in clinical trials represents an unprecedented opportunity to explore the relationship between histologic tumor type, defined molecular abnormalities, and response to drugs with targeted mechanisms of action. We currently do not have enough data about the functional consequence of many mutations, nor on how those functional consequences differ across different histologic tumors or in tumors with additional molecular abnormalities. The evidence that a particular mutation is a "driver"—and that its activity can be modulated by a given treatment is very sparse—making it difficult to design clinical trials with appropriate levels of evidence that a tumor with the molecular abnormality could be impacted by a given treatment. The NCI-MPACT (Table 3) trial is attempting to improve knowledge in this area and is randomizing patients, after a tumor biopsy and NGS assay, to a treatment that is hypothesized to be effective (Arm A) or to the complementary set of treatments (Arm B). If the results demonstrate a benefit for the patients treated on Arm A, this will provide some evidence for the usefulness of NGS in early phase trials. If no difference is observed between the arms, it may mean that the molecular eligibility criteria chosen were not drivers, the drugs were not effective, or that patients will do as well with any cancer treatment targeted to a general cancer vulnerability.

Current trial designs focus on finding efficacy signals, but next steps may be challenging for low prevalence driver mutations. In NCI-MATCH (32), for example, patients with refractory solid tumors or lymphomas will be screened for actionable mutations, translocations, or amplifications and an array of drugs or drug combinations will be available to which patients can be assigned. As these sub-protocols will not specify a particular histologic tumor type, the goal is to find a signal of activity across tumor types. Follow-up trials or expansion cohorts will then be needed to explore that signal further. However, as most mutations occur in a prevalence of 2% to 10%, a large screening effort and widespread availability in the community will be critical in order to implement such trials.

It is also likely that genomics by itself will be inadequate to characterize the molecular signaling landscape of a tumor. Technical advances have brought transcriptome sequencing assays to the research space for use in tissues that are formalin fixed and paraffin embedded, but additional validation will be needed to bring these to the clinic. Likewise, protein expression may well be important, and more robust methods to measure this are needed. Robust, validated assays that can be used in the clinic are necessary to assess the predictive characteristics of epigenetic and mRNA abnormalities. The state of the immune system may be crucial for understanding response to chemotherapy, and potentially to

agents that inhibit cancer signal transduction as well as to immune-modulating agents. There are also interactions between the genomic landscape in cancer and responses to immunotherapy, supporting the integration of these therapeutic areas (33). None of these types of knowledge and technologies by themselves are likely to give a total picture of a tumor biopsy, and there are not presently robust and clinically validated methods to incorporate all into a coherent picture for prediction of response. A systems biology-based approach to identify master regulators that control functional tumor dependencies arising from multiple molecular events may be relevant to integrate information obtained from several technologies and platforms (34). Computational biology and bioinformatics will play a crucial role to assimilate and analyze a large volume of clinical and molecular data to enable appropriate treatment decisions.

In addition, the impact of tumor heterogeneity has yet to be addressed, both within primary tumors and across metastases (4, 35, 36). At the present time, tumor tissue is necessary to perform genomic profiling, and some trials use archived tumor, although some trials require a biopsy at the time of study entry. As noted above, both archived samples and fresh tumor biopsies have drawbacks, and both methods are subject to sampling bias. With advancements in technology, there is potential that ctDNA may substitute for a biopsy, but this has yet to be proven. Despite these hurdles, there are enough successes to continue to refine and streamline genomically enabled clinical trials. As we work toward this goal, lessons learned may assist in incorporating additional technologies to improve our ability to predict the course of a malignant disease and to intervene intelligently to improve and prolong life for patients with cancer. The general dissemination of genomic profiling for the purpose of treatment recommendation can be resource and time intensive, and can be potentially harmful if patients forgo established treatment outside of clinical trials. As such, it is crucial for the cancer community to support genomic-based clinical trials to develop the evidence needed for this precision medicine–based approach to therapy.

Disclosure of Potential Conflicts of Interest

P.M. LoRusso reports receiving speakers bureau honoraria from Genentech and is a consultant/advisory board member for Astex Pharmaceuticals, Celgene, Genentech, and Pfizer. No potential conflicts of interest were disclosed by the other authors.

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